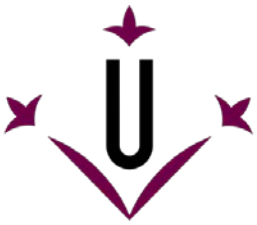

# Growth environment but not seed position on the parent plant affect seed germination of two *Thlaspi arvense* L. populations

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**ABSTRACT**

*Thlaspi arvense* L. is a common weed found in most temperate regions throughout the world that also shows excellent potential for domestication as an oilseed crop. The complexity of *T. arvense* seed dormancy presently makes it difficult to manage as a weed or oilseed crop. Therefore, a better understanding of factors controlling seed dormancy will help to develop strategies for managing its weediness and facilitate development of crop genotypes. *T. arvense* has semi-indeterminate growth and seeds developed at the base of the inflorescence (basal) generally mature sooner than those near the top (apical). However, little is known about the maternal influences on the level of dormancy and germination of basal and apical seeds. To study this, two *T. arvense* populations, one from Spain and the other from USA, were both grown at field sites in Almenar, Spain and Morris, Minnesota, USA during the same growing season (autumn 2012-summer of 2013). Basal and apical seeds collected at maturity were analyzed for mass, total protein and carbon content, oil content, and fatty acid profiles. Under controlled environment conditions, seed germination was measured at 5, 8, 11, 14, 17, and 20°C and germination rate estimated at 8°C. Seed position on the inflorescence did not affect germination consistently nor were there clear differences in seed attributes between positions. However, seeds of both populations produced at Almenar had lower germination and were consistently larger and contained higher oil and carbon content than seeds produced at Morris. Results indicate that environmental influences at each growth location played a much larger role in influencing dormancy and germination of seed produced than did the seed attributes measured or position of seed on the inflorescence.

*Keywords: Thlaspi arvense*; Pennycress; Germination; Seed dormancy; Seed oil

## 1. Introduction

*Thlaspi arvense* L., often referred to as field pennycress, is recognized by many as a weed (Warwick et al., 2002). Recently, however, *T. arvense* has gained considerable attention as a winter oilseed cover crop for double cropping systems with soybean [*Glycine max* (L.) Merr.] as the primary crop in the Upper Midwest of the USA (Phippen and Phippen, 2012; Johnson et al., 2015). Moreover, efforts are currently underway to domesticate *T. arvense* as a viable oilseed crop. The genome of *T. arvense* has been sequenced recently (Dorn et al., 2013), and the genomics tools developed are being used in breeding programs to improve its agronomic and end-use characteristics (Sedbrook et al., 2014). Seed oil of *T. arvense* lends itself well to making biodiesel and renewable aviation fuel (Moser et al., 2009; Fan et al., 2013).

*T. arvense* is a wide spread weed species, commonly found in most temperate regions in the northern and southern hemispheres (Warwick et al., 2002). *T. arvense*'s success as a weed is in part due to its ability to withstand climatic extremes of temperature and drought, as well as the complex nature of primary and secondary dormancy of its seeds, and thus, the ability of its seed bank to persist for extended periods (Hazebroek and Metzger, 1990a). Gaining a better understanding of environmental and maternal influences affecting its seed dormancy, and hence, germination characteristics will help to develop strategies for weed management and provide necessary information for developing domestic genotypes lacking dormancy (Sedbrook et al., 2014).

Both growth environment (Saarinen, et al., 2011) and influences on developing seed by the parent plant (Hume, 1994; Donohue et al., 2007) can impart various degrees of seed dormancy and affect germination. With regards to maternal influences, seed traits such as large seed size (Moles et al., 2000) and high oil content (Gardarin et al., 2011) have been linked generally to

greater seed germination. *T. arvense* is semi-indeterminate with respect to flowering and seed set, with seed development initiating from the bottom (basal) and progressing to the top (apical) of the inflorescence similar to the reproductive phenology of *Camelina sativa* L. (Martinelli and Galasso, 2011). Few, however, have studied the differences in physiochemical characteristics between apical and basal seeds of *T. arvense* and whether they affect the degree of dormancy and germination of the seed.

We hypothesized that because of the temporal variation in the development between apical and basal seed on the inflorescences of *T. arvense*, the physiochemical attributes of the seed will be affected and therefore, will influence the level of dormancy and germination between the seed types. To test this hypothesis, two different *T. arvense* populations (i.e., accessions), one originating from northeastern Spain and the other from north-central USA, were both grown at field sites in Almenar, Spain and Morris, Minnesota, USA during the same growing season (autumn 2012 to summer of 2013). Basal and apical seeds were collected from plants at maturity and were analyzed for mass and biochemical attributes. In a separate controlled environment experiment, the germination of these seeds was measured at 5, 8, 11, 14, 17, and 20°C and germination rate estimated at 8°C.

## 2. Materials and methods

### *2.1 Cultural practices, site description*

*T. arvense* seeds used for the study were harvested from mature plants between June and July 2012 in Morris, Minnesota USA (45º43’36’’N-95º49’17’’W) and Camerillas, Teruel, Spain (40º38’39’’N-0º48’’35’’W). Seeds were stored dry under laboratory conditions after harvest

until they were sown for the study in early autumn. Portions of the two seed lots were interchanged and both seed populations were sown for the study at each field site. The field site in Spain was at Almenar (41º46'36''N-0º32'7''E) and in USA was at Morris (same as above).Spring wheat (*Triticum aestivum* L.) was the previous crop at both sites.

Seeds were sown in 1 $m^2$ plots in a randomized complete block design with four replicates. The treatment variable was seed origin (Spain or USA). In each plot, 1000 seeds were sown at a depth of 1 cm in four rows (250 seed per row) spaced 25 cm apart to simulate cultivation conditions and to facilitate hand-weeding of the plots. This also helped to identify and remove any volunteer seedlings. Sowing dates were 18 September 2012 for Morris and 4 October 2012 for Almenar.

When plants had fully matured (pods were dry and brittle and seeds were a dark reddish-brown color), from 10 to 25 randomly chosen plants were harvested from each plot and brought to the laboratory for processing. Plants in Almenar were harvested 3 June 2013 and in Morris they were harvested 9 July 2013. For each plant harvested, five to seven pods from the bottom (basal seed) and five to seven pods from the top (apical seed) of the main raceme were sampled, their seeds collected, and the 1000-seed weights determined for each plot.

*2.2 Seed chemical analyses*

For chemical analyses, because the individual (replicate) seed samples were relatively small, for each population and growth location, all four replicates of basal seed were combined (i.e., pooled) as were those of the apical seed. All chemical analyses of seed were done in triplicate by sub-sampling the pooled seed of apical and basal seeds. Approximately 200 mg of finely ground seed was used to determine total N and C using a Leco CN-2000 combustion analyzer (Leco

Corporation, St. Joseph, MI) and the seed N content was multiplied by 6.25 to convert to estimated protein content (Gesch et al., 2014). Approximately 0.5 to 2.0 g of whole seed were used to determine total oil content non-destructively by pulsed NMR using a Bruker Minispec pc120 (Bruker, The Woodlands, TX). The instrument was calibrated with pure *T. arvense* oil. Additionally, approximately 50 mg of ground seed was extracted with hexane to make fatty acid methyl esters (FAME), which were then used to determine fatty acid profiles of the seed oil by gas chromatography (Hewlett-Packard 5890 Series II, Palo, Alto, CA) equipped with a flame-ionization detector and an auto-sampler/injector using the methods previously described by Forcella et al. (2005).

*2.3 Germination experiment*

The basal and apical seeds that were collected from the field experiment (2012-2013) were used to conduct germination tests at Lleida, Spain during 2013 and 2014. Seed germination was tested in controlled environment chambers at constant temperatures of 5, 8, 11, 14, 17, and 20°C in the dark. At each temperature, for each pennycress population and seed type (basal and apical), 4 replicates of 25 seeds each were tested. The seeds were placed in Petri dishes on a medium of 14% agar and the number of germinated seeds was counted daily for 21 d under a green light. The germination experiment under all six temperatures was repeated three times for seeds collected from plants grown in Almenar and twice for seeds collected in Morris. The data are presented as mean total percent germination after 21 d, and data collected for the 8°C treatment (found to be generally most optimal for germination) were used to determine germination rate by analyzing germination as a function of time using a Boltzmann sigmoidal function of the form:

$Y = A/\{1+\exp[2*\ln(9)*(G_{50}-GDD)/B]\}$

where $Y$ is percent seed germination, GDD is accumulated growing degree days, $A$ is percent maximum germination, $G_{50}$ is growing degree days from the start of the experiment to 50% of maximum germination, and $B$ is growing degree days from 10 to 90% of maximum germination (Gesch and Archer, 2005). Previously, a first estimation of the base temperature ($T_b$) was performed for each seed type, as described by Bradford (2002). This estimation was performed by calculating the inverse of the time (days) needed for 50% of germination at each of the six constant temperatures. The points were then regressed against temperature and intersect with 0 was used as $T_b$ for calculating the GDD for each seed type.

*2.4 Statistical analysis*

Data of percent germination and chemical composition of seed were analyzed by 2-way ANOVA for each of the two pennycress populations studied using the General Linear Model Procedure of SAS (SAS for Windows 9.1, SAS Inst., Cary, NC). The main effects and their interaction used in the model were location (i.e., Almenar and Morris) where the seed was produced and position of seed (i.e., basal and apical) on the plant. When effects were significant at the $P \leq 0.05$ level, mean comparisons were made using Fisher's LSD test. Germination rate was estimated using JMPro11 (SAS Inst., Cary, NC).

## 3. Results

*3.1 Climate*

Between sowing and seed collection of *T. arvense*, the overall average air temperature at the Morris location was 4.1°C compared to 12.5°C at Almenar for the same period (Table 1). During

the winter months (November to March), the temperature averaged 12.3°C lower in Morris compared to Almenar. The number of accumulated GDD from sowing to harvest was 1441°C d at Morris and 1904°C d at Almenar. Although the total amount of precipitation received at both sites was not greatly different, the pattern of accumulation was (Table 1). For instance, only 16% of total precipitation at Morris fell between September and January, and 84% fell between February and July. In contrast, total precipitation at Almenar was divided evenly between the same two periods (Table 1).

*3.2. Seed germination and germination rate*

No clear pattern of total germination distinguished apical from basal seeds of the Spanish population (Fig. 1 A and C). Apical seed of the Spanish population grown at Almenar had higher germination than basal seed at 5, 8, and 17°C, but when grown at Morris, their basal seed had higher germination than apical seed at 17 and 20°C. For the USA population, there was a tendency for apical seed to have greater germination than basal seed (Fig. 1 B and D). However, for this population grown at Almenar, differences between apical and basal seeds were not significant at any temperature, whereas apical seed germination was higher than basal seed at 5, 11, and 14°C from plants grown at Morris.

One clear pattern that developed, was that seeds from plants of both populations grown at Morris always had higher germination rates than those from plants grown at Almenar at all temperatures except for the apical seeds of the USA population at 5°C (Fig. 1 A-D). Also, for plants grown at Morris, across all temperatures tested, the seeds of the Spanish population tended to have higher germination rates than the USA population (Fig. 1 C and D; $P \leq 0.05$), which may reflect seed size differences (see below).

In general, the rate of germination at 8°C, as determined by the parameters $G_{50}$ (i.e., accumulated GDD from initiation to 50% of maximum germination) and *B* (i.e., GDD from 10 to 90% of maximum germination) of the Boltzmann sigmoid function, were similar between apical and basal seeds of both populations regardless of growing location (Table 2). However, for the Spanish population grown in Morris, apical seed did have a lower $G_{50}$ (69 GDD) than basal seed (85 GDD) indicating a more rapid initiation of germination, although the rate of germination following initiation, as determined by *B*, did not differ (Table 2). As expected, there were differences in parameter *A* (i.e., maximum germination) in several instances between seed position and populations, which are also indicated in the results shown in Figure 1. In comparison between locations, when averaged across both populations and seed position, seeds produced in Morris generally had lower *B* (43 GDD) and $G_{50}$ (79 GDD) values than those produced in Almenar (*B* 59 and $G_{50}$ 90), indicating a slightly faster germination rate in Morris than in Almenar regardless of seed origin.

*3.3 Seed physiochemical attributes*

Seed mass was not different between apical and basal seed of the Spanish population, but it was different for the USA population where basal seed was heavier at both locations (Table 3). For both populations, seed mass was generally greater for seeds produced in Almenar Spain. In comparison, the Spanish-origin seeds tended to be heavier than seed of the USA population regardless of growing location (Table 3). Averaged across locations, the Spanish seeds were 1.25 g per 1000-seeds, whereas the USA seeds weighed 0.97 g per 1000-seeds ($P \leq 0.05$).

Seed oil content varied between apical and basal seed of the Spanish population at Morris and Almenar but was consistently higher in basal seed for the USA population (Table 3).

Although differences in oil content between apical and basal seed were generally significant, the differences were rather marginal, whereas differences between growing locations were more substantial.

Seed oil content of both populations was greater for seeds produced in Almenar, consistently > 32% (Table 3). When grown in Morris, however, seed oil dropped to 27% for Spanish seeds and 31% for USA seeds. When averaged across growing locations, the USA seed had higher oil content (31.5%) than the Spanish seed (29.4%; $P \leq 0.05$).

Protein and carbon content did not differ between apical and basal seed of the Spanish population at either growing location but both were greater in basal compared to apical seed of the USA population (Table 3). Overall, protein content of the Spanish population was greater for seeds produced in Morris but there was no difference between locations for the USA population. In comparison of the two populations, when averaged across locations, seeds of the Spanish population generally had greater protein and lower carbon content than seeds of the USA population ($P \leq 0.05$).

The fatty acid (FA) profile of seed oil differed slightly between apical and basal seed of the Spanish population (Table 4). But again, although significant, the differences in FAs between seed position were generally marginal. More intriguing, however, were differences in FA distribution for seeds produced between locations. For the Spanish population, seeds produced in Almenar contained a higher degree of FA unsaturation (i.e., C18:2 and C18:3) than seeds produced in Morris. Conversely, the Morris-grown seeds had greater saturation of FAs (i.e., C16:0, C18:1, C20:1, and C22:1) than those from Almenar (Table 4). There were no differences in FA distribution between apical and basal seed for the USA population grown at either location. However, there was again a difference in saturation in FAs between the two locations

with the seed produced in Morris having a greater level of saturation (Fig. 2). In comparison, the two different populations had relatively similar FA profiles. However, the Spanish population when averaged across both growing locations tended to have a greater erucic acid (C22:1) content (35.7%) than the USA population (33.2%). This came at a cost of its C18:1 and C18:2 FA content, which were about 1 to 2% lower than that of the USA population seed.

## 4. Discussion

In the present study, seed position on the stem did not have a clear effect on seed germination for either of the *T. arvense* populations regardless of growth location. This disproved our hypothesis that basal and apical seeds would differ in their degree of germination due to differences in the accumulation of seed reserves resulting from temporal differences in seed development on the inflorescence. Nevertheless, there was a clear pattern of substantially higher seed germination at all temperatures tested for both populations grown in Morris compared to seeds produced in Almenar. Furthermore, seeds of both populations produced in Morris generally had faster germination rates than those from Almenar. Lower total germination and slower germination rate are indicative of dormancy in weed seeds (Colbach et al., 2006; Gardarin et al., 2011), and our results indicate that seeds from Almenar developed a greater degree of dormancy. The question then becomes how and why?

With regard to the relationship between germination and physiochemical attributes of the seed tested, certain associations were found. Seed mass, oil content, and total carbon content were always greater in the seeds produced at Almenar. Seed size and shape can influence germination and dormancy among weed species (Moles et al., 2000) and within a species (Baloch et al., 2001). Studying a relatively wide range of seed size (< 6 to > 11 mg) from a single

population of *Abutilon theophrasti* Medik., Balock et al. (2001) found that germination was greatest for seed that ranged from 8.0 to 9.9 mg and became lower with seeds of higher or lower mass.

Lipids have a higher energy content than other primary storage reserves found in seeds, such as protein and sugars, and they have been linked to variations in germination and dormancy of a wide range of weed species (Bretagnolle et al., 2015; Gardarin et al., 2011; Linder, 2000). High seed oil content typically has been associated with greater germination and germination rate (Gardarin et al., 2011), although its potential involvement in dormancy is not fully clear (Gardarin and Colbach 2014; Okagami and Terui, 1996). In the case of *T. arvense*, our results showed that higher oil content was associated with low germination and greater dormancy. Also intriguing was the difference that we found in the saturation of oil between seeds produced in Almenar and Morris. Differences in the metabolism of various fatty acids during the germination process may affect germination rate and even total germination (Okagami and Terui, 1996). Linder (2000) proposed that the lower melting point of unsaturated compared to saturated fatty acids, allows seeds with high unsaturated FA levels to germinate earlier and grow faster at low temperatures. Results from the present study, however, do not appear to fit this theory, as the seeds of both *T. arvense* populations produced in Almenar had higher unsaturation FA levels in their seeds [i.e., (C18:2 + C18:3)/(C16:0+C18:1+C20:1+C22:1)] than those produced in Morris, but also had lower and slower germination. However, it should also be noted that saturated fatty acids (i.e., C16:0, C18:0, and C20:0) in the seed oil of both populations regardless of growth location were generally low, only making up 3 to 4% of the total oil.

The differences observed in germination and dormancy of *T. arvense* from Almenar and Morris were far more likely due to environmental conditions experienced (Gulden et al., 2004;

Saarinen et al., 2011) or maternal influence (Hume, 1994; Donohue et al., 2007) during maturation and seed development. Conditions were generally drier and cooler during seed development at Almenar, which might have impacted their germination. Both temperature and dry conditions have been shown to influence dormancy in *T. arvense* seed (Hazebroek and Metzger, 1990a; Hazebroek and Metzger, 1990b). Plants grown at Almenar, most of which emerged in autumn (Royo-Esnal et al., 2015), flowered in April, and their seed matured in May, while at Morris, most plants emerged in the spring, began flowering in late May, and matured seed in June. During May and June, plants at Morris received much more precipitation (240 mm) than did plants at Almenar (93 mm) during April and May when they were flowering and setting seeds (Table 1). Also, temperatures were warmer at Morris during seed development than Almenar (Table 1). Lastly, daylength during seed maturation in Almenar (May) and Morris (June) averaged 14.3 and 15.4 hr, respectively (Table 1), and may have influenced oil contents and FA profiles (Seiler, 1986).

Depending on environmental conditions, *T. arvense* can act as a winter or spring annual (Baskin and Baskin, 1989; Warwick et al., 2002). Saarinen et al. (2011) found that *T. arvense* plants that emerged in the autumn and experienced a period of warming during the winter tended to impart a greater degree of dormancy on seeds that they produced compared to seeds produced by plants that emerged in the spring. This to a large degree may explain the results for germination that we observed for seeds produced from plants in Almenar and Morris. At Almenar, the proportion of the USA population that emerged in autumn was 87% with 13% emerging in the spring, while for the same population in Morris, only 9% emerged in autumn and 91% in the spring (Royo-Esnal et al., 2015), and results were nearly identical for the Spanish population.

Also, the length of time for plants to mature may have played a role in imparting dormancy. Others have also shown that the maternal environment can affect growth of plants and the dormancy of their offspring (Hume, 1994; Donohue et al., 2007). For instance, Hume (1994) found that seeds produced by a late-flowering strain of *T. arvense* germinated faster than seeds collected from an early-flowering strain. In the present study, both *T. arvense* populations grown at Almenar matured over a longer period, flowered earlier, and accumulated more GDD (Table 1) than plants at Morris, in part due to Almenar's milder winter. This is the most likely reason why Almenar-grown seeds were generally much larger and contained more oil and total carbon content than Morris-grown seeds. Our results corroborate those of Saarinen et al. (2007), who also found that seeds produced by autumn-emerging plants were generally larger than those from plants that emerged in spring, and also had lower germination.

Moreover, some authors confirm for other species that seeds produced in locations with more daylight hours present lower dormancy than those produced in locations with less daylight hours (Baskin & Baskin, 1998). Daylength was longer in Morris (15.4 h in June) than Almenar (14. 3 h in May) when seeds were maturing on plants, which emphasizes this theory.

## 4. Conclusions

The germination and degree of dormancy of seeds produced from two *T. arvense* populations, one from Teruel, Spain and the other from Minnesota, USA, which were both grown at each field site in Almenar, Spain and Morris, Minnesota, appear to have been affected more greatly by growth environment and maternal influence during development than by physiochemical attributes of the seeds themselves. Furthermore, we did not see a clear relationship in differences in germination amount or rate between apical and basal seeds, which temporally differ in their

maturity on the plant stem. These results help gain a better understanding of conditions affecting the degree of dormancy imparted on *T. arvense* seed during their development. This will help to make better predictions of seedling emergence dynamics from native seed populations, and perhaps help target traits to modify to reduce or eliminate dormancy in efforts to domesticate *T. arvense* as a commercial oilseed crop (Sedbrook et al., 2014).

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**Figure Legends**

Figure 1. Total percent germination of apical and basal seeds at six different temperatures for seeds of Teruel Spain (A and C) and Minnesota USA (B and D) *Thlaspi arvense* populations grown at Almenar Spain (A and B) and Morris, Minnesota USA (C and D). Bars at each temperature for a given graph followed by a different letter are significantly different at the $P \leq 0.05$ level. Values are means ± SE.

Figure 2. Differences in the primary fatty acids as a percent of total seed oil for seeds of the Minnesota USA *Thlaspi arvense* population produced at Morris, Minnesota USA (black bars) and Almenar Spain (gray bars). For each fatty acid, bars followed by different letters are significantly different at the $P \leq 0.05$ level. Values are means across both apical and basal seeds ± SE.

Table 1. Monthly climate data for Almenar Spain and Morris, Minnesota USA during propagation of *Thlaspi arvense* plants from sowing to harvest during 2012-2013.

| | Morris, Minnesota USA | | | | Almenar Spain | | | |
|---|---|---|---|---|---|---|---|---|
| Month | Mean temperature (°C) | Accumulated GDD (°C d) | Accumulated precipitation (mm) | Mean daylength (h) | Mean temperature (°C) | Accumulated GDD (°C d) | Accumulated precipitation (mm) | Mean daylength (h) |
| Sept | 15.5 | 349 | 0 | 12.3 | 19.6 | 479 | 49 | 12.2 |
| Oct | 7.0 | 112 | 29 | 10.6 | 15.2 | 362 | 92 | 10.8 |
| Nov | 0.2 | 15 | 16 | 9.3 | 9.2 | 166 | 30 | 9.6 |
| Dec | -8.3 | 2 | 5 | 8.6 | 5.6 | 73 | 7 | 9.1 |
| Jan | -10.8 | 0 | 11 | 9.0 | 4.6 | 35 | 31 | 9.4 |
| Feb | -10.3 | 0 | 0 | 10.1 | 5.4 | 60 | 8 | 10.4 |
| March | -6.8 | 0 | 33 | 11.7 | 9.2 | 169 | 68 | 11.7 |
| April | 1.8 | 38 | 16 | 13.3 | 11.7 | 237 | 78 | 13.1 |
| May | 13.1 | 292 | 61 | 14.7 | 13.0 | 284 | 14 | 14.3 |
| Jun | 19.3 | 456 | 179 | 15.4 | 19.2 | 469 | 37 | 15.0 |
| Jul | 24.1 | 177 | 21 | 15.1 | 24.7 | 192 | 5 | 14.7 |
| Total | | 1441 | 371 | | | 2526 | 419 | |

Table 2. Estimated $T_b$ for the calculation of GDD and Boltzmann function parameters for seed germination rates of the Spain and USA *T. arvense* populations grown in Almenar, Spain, and Morris, USA germinated at 8°C. Values are means ± SE. Different letters denote significances at P<0.05. The first lower case letters denote differences between apical and basal seeds within a population and growth location; upper case letters denote differences between the same seed position of different populations at the same growth location; and the second lower case letters denote differences between the same seed position of the same population grown at different locations.

| Location grown | Population | Seed position | $T_b$ (°C) | *A* | *B* | $G_{50}$ | Model P<0.01 |
|---|---|---|---|---|---|---|---|
| Almenar | Spain | Apical | +3.0 | 4.3 ± 0.2 -Ab | 44 ± 13 -Aa | 83 ± 3 -Aa | ** |
| | Spain | Basal | +2.4 | - | - | - | n.a.† |
| | USA | Apical | +1.7 | 2.7 ± 0.2 aBb | 74 ± 19 aAa | 88 ± 5 aAa | ** |
| | USA | Basal | +2.0 | 2.1 ± 0.3 a-b | 60 ± 27 a-a | 93 ± 8 a-a | ** |
| Morris | Spain | Apical | +1.3 | 21.1 ± 0.2 bAa | 38 ± 3 aAa | 69 ± 1 bAb | ** |
| | Spain | Basal | +1.6 | 22.3 ± 0.4 aA- | 42 ± 4 aA- | 85 ± 1 aA- | ** |
| | USA | Apical | +2.5 | 14.9 ± 0.4 aBa | 49 ± 9 aAa | 82 ± 2 aAa | ** |
| | USA | Basal | +2.1 | 12.5 ± 0.4 bBa | 43 ± 7 aAa | 78 ± 2 aAb | ** |

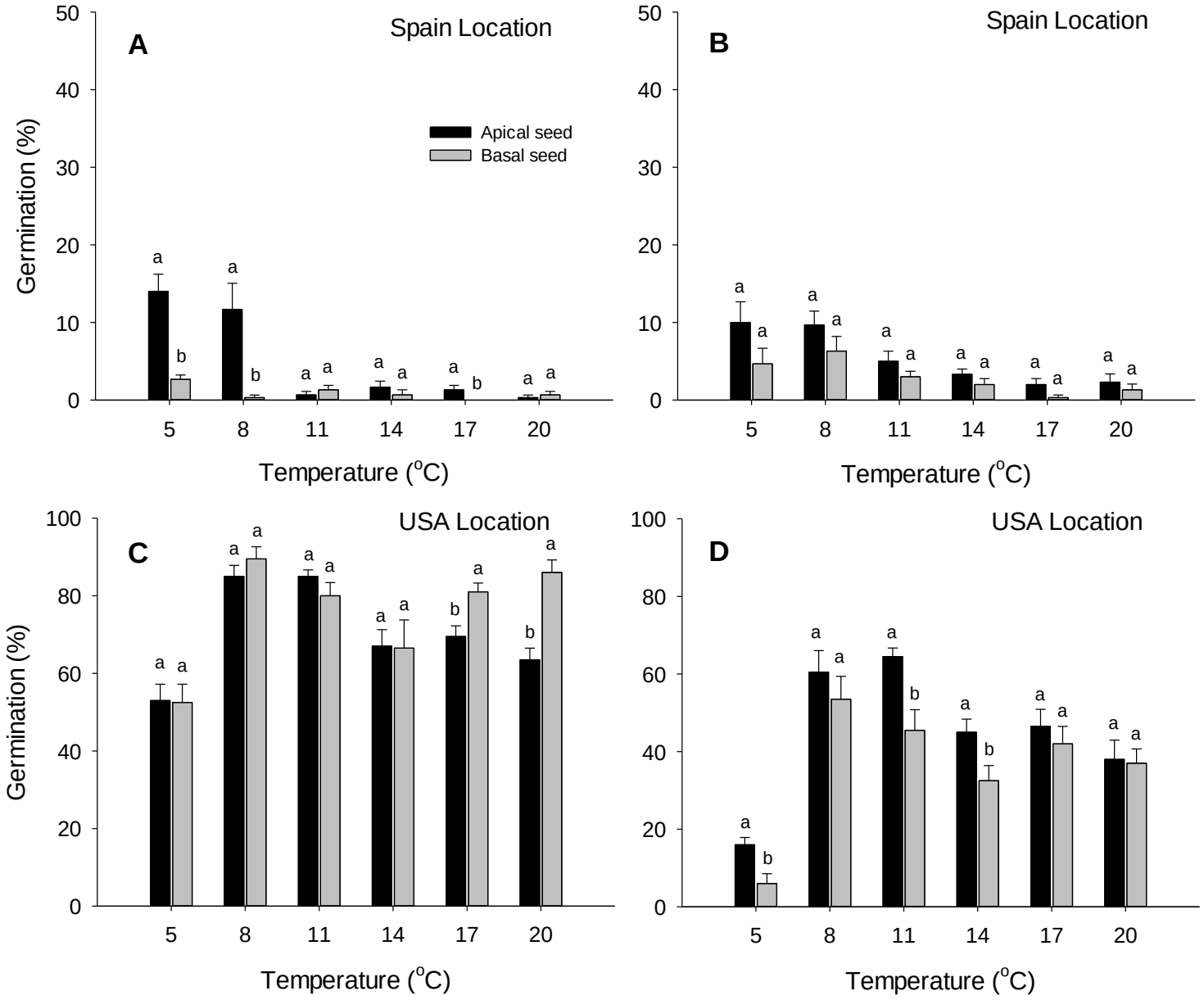
†Abbreviation: n.a., not applicable.

Table 3. Oil, crude protein, and carbon content of apical and basal seeds for the Spain and USA *Thlaspi arvense* populations that were grown at Almenar and Morris during 2013. Comparisons in the upper half of the table are between apical and basal seeds for each growing location. Comparisons in the lower half of the table are for location and seed position over both locations for a given attribute. Values are means, and the SE is included for apical and basal seed for each growth location. Comparisons within columns followed by a different letter are significant at the $P \leq 0.05$ level.

| | | *T. arvense* (Spain population) | | | | *T. arvense* (USA population) | | | |
|---|---|---|---|---|---|---|---|---|---|
| Location Grown | Seed position | Seed mass (g/1000 seed) | Oil (%) | Protein (g $kg^{-1}$) | Carbon (g $kg^{-1}$) | Seed mass (g/1000 seed) | Oil (%) | Protein (g $kg^{-1}$) | Carbon (g $kg^{-1}$) |
| Morris | Apical | 1.01 ± 0.04 a | 26.9 ± 0.04 a | 286 ± 1 a | 564 ± 2 a | 0.74 ± 0.01 b | 30.5 ± 0.03 b | 262 ± 3 b | 578 ± 1 b |
| | Basal | 1.12 ± 0.03 a | 26.4 ± 0.09 b | 287 ± 7 a | 562 ± 4 a | 0.88 ± 0.01 a | 30.8 ± 0.08 a | 272 ± 3 a | 584 ± 2 a |
| Almenar | Apical | 1.44 ± 0.02 a | 32.1 ± 0.02 b | 267 ± 3 a | 587 ± 2 a | 1.14 ± 0.02 b | 32.2 ± 0.04 b | 266 ± 1 b | 588 ± 1 b |
| | Basal | 1.40 ± 0.03 a | 32.3 ± 0.04 a | 267 ± 2 a | 585 ± 2 a | 1.28 ± 0.04 a | 32.6 ± 0.04 a | 278 ± 3 a | 594 ± 2 a |
| Comparison by | | | | | | | | | |
| Location | Morris | 1.07 b | 26.7 b | 287 a | 563 b | 0.81 b | 30.6 b | 267 a | 581 b |
| | Almenar | 1.42 a | 32.2 a | 267 b | 586 a | 1.12 a | 32.4 a | 272 a | 591 a |
| Seed position | Apical | 1.28 a | 29.5 a | 277 a | 575 a | 0.96 b | 31.3 b | 264 b | 583 b |
| | Basal | 1.29 a | 29.3 b | 276 a | 574 a | 1.10 a | 31.7 a | 275 a | 589 a |

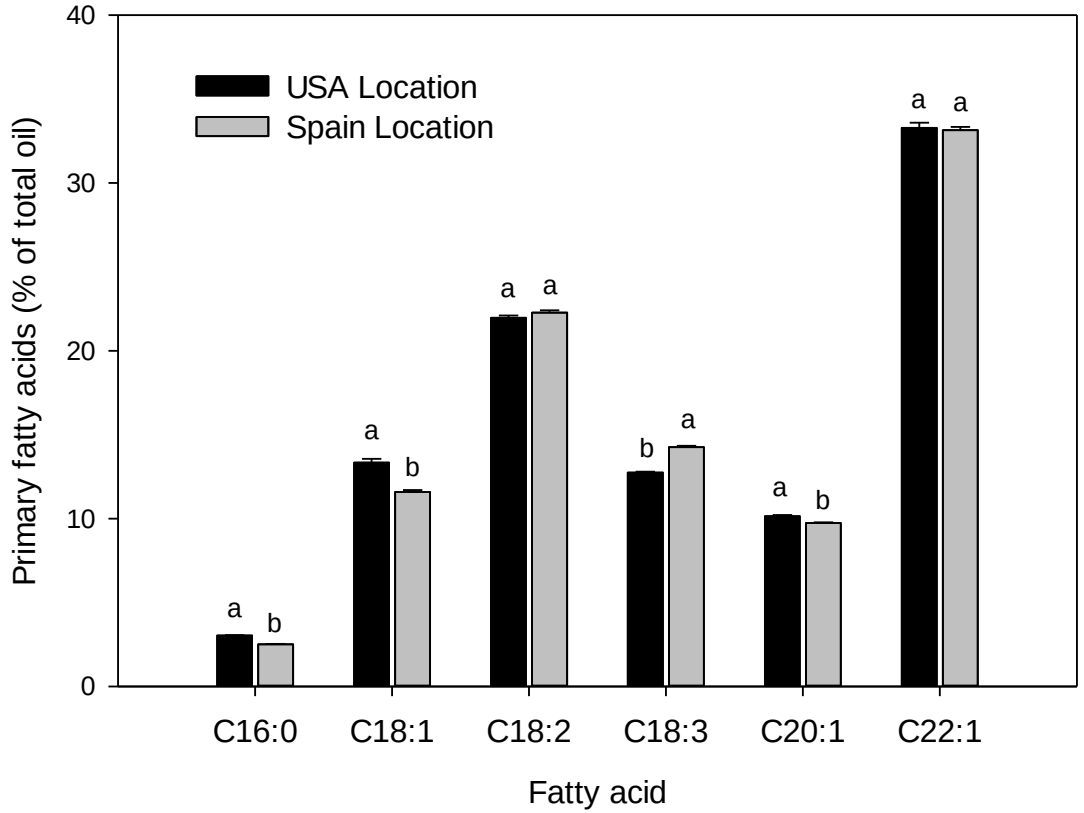
Table 4. Fatty acid distribution of seeds for the Spain *Thlaspi arvense* population that was grown in Morris, MN USA and Almenar Spain during the 2012-2013 growing season. Values are means ± SE. For seed position and location within a column, values followed by a different letter are significantly different at the $P \leq 0.05$ level.

| | Seed fatty acids | | | | | |
|---|---|---|---|---|---|---|
| Seed Position | C16:0 | C18:1 | C18:2 | C18:3 | C20:1 | C22:1 |
| Apical | 3.0 ± 0.16 a | 11.2 ± 0.26 b | 20.4 ± 0.29 a | 13.6 ± 0.33 a | 9.4 ± 0.03 a | 35.5 ± 0.28 b |
| Basal | 2.8 ± 0.16 b | 11.8 ± 0.30 a | 20.1 ± 0.20 a | 13.3 ± 0.25 b | 9.2 ± 0.14 b | 35.9 ± 0.07 a |
| | | | | | | |
| Location | C16:0 | C18:1 | C18:2 | C18:3 | C20:1 | C22:1 |
| Morris | 3.3 ± 0.03 a | 12.1 ± 0.16 a | 19.8 ± 0.09 b | 12.8 ± 0.07 b | 9.4 ± 0.05 a | 36.0 ± 0.08 a |
| Almenar | 2.6 ± 0.02 b | 10.9 ± 0.14 b | 20.6 ± 0.25 a | 14.1 ± 0.14 a | 9.1 ± 0.11 b | 35.3 ± 0.24 b |


A
Spain Location
Apical seed
Basal seed
Germination (%)
Temperature (°C)
B
Spain Location
Temperature (°C)
C
USA Location
Germination (%)
Temperature (°C)
D
USA Location
Temperature (°C)

445

446 Fig. 2


USA Location
Spain Location
Primary fatty acids (% of total oil)
40
30
20
10
0
a b
a b
a a
b a
a b
a a
C16:0
C18:1
C18:2
C18:3
C20:1
C22:1
Fatty acid

447